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DIETARY EFFECTS ON THE UPTAKE OF BENZO[*a*]PYRENE

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Abstract—It has been established that exposure to polycyclic aromatic hydrocarbons (PAHs), or more specifically benzo[*a*]pyrene (B[*a*]P), either by inhalation through cigarette smoking or by contact through occupational exposure of the lungs or skin, can result in cancerous lesions. It appears that the general population consumes more B[*a*]P from food than from smoking. Despite this, epidemiological studies have not implicated B[*a*]P from foods as a causative factor in some human cancers. This lack of an epidemiological correlation between cancer incidence and intake of dietary PAHs/B[*a*]P could be due to some 'protective' or 'detoxification' mechanism. Despite the abundance of literature regarding the food content of B[*a*]P, there are few data concerning its uptake from foods. In the present study we investigated the intestinal absorption of B[*a*]P from foods using bile duct cannulated rats and radioactive B[*a*]P. [^{14}C]B[*a*]P was first added to solvents such as water, corn oil, liquid paraffin or 50% ethanol, which were then administered by gavage to rats fed diets with or without added carbon. Additionally, food polyphenols such as quercetin and chlorogenic acid were also tested for their effect on the absorption of B[*a*]P. The results indicated that the excretion of B[*a*]P in the bile was reduced by water, carbon, quercetin and chlorogenic acid but was potentiated by corn oil. To complement the *in vivo* studies, some *in vitro* tests to investigate the efficiency of B[*a*]P extraction from different foods using water or oil as solvents were also performed. These tests indicated that extraction of B[*a*]P from foods was affected by the solvent. It is postulated that reduced solubility, physical adsorption and the formation of chemical adducts between B[*a*]P and some food ingredients, play a sporadic, although still not well determined, role in reducing the absorption of B[*a*]P from the gut. The results of these studies suggest that B[*a*]P absorption from the intestinal tract is markedly affected by dietary components, and that this may be a factor that contributes to the lack of an epidemiological correlation between some human cancers and the B[*a*]P content of foods.

INTRODUCTION

In addition to the nutritional aspects of the diet, food may contain components that are nutritionally and biologically inert, or may even produce toxic effects. These undesirable compounds could be either of natural origin, or result from the production, storage or processing of foods (Knudsen, 1986; Pariza, 1989).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds appearing in food, the workplace and the biosphere (Howard and Fazio, 1980). Many of them were found to be potent mutagens in a variety of short-term *in vitro* tests, including the Ames Salmonella assay (Levin *et al.*, 1978). There are a number of studies that clearly indicate that exposure to PAHs, and more specifically benzo[*a*]pyrene (B[*a*]P), whether inhaled by smoking or contacted through occupational exposure, will lead to the development of cancerous or precancerous lesions in the lungs or on the skin (Grimmer, 1985; Harvey, 1985). Other studies, using experimental laboratory animals, have provided evidence that larger doses of orally administered B[*a*]P can induce neoplastic lesions in several organs (Griciute, 1979).

Hattermer-Frey and Travis (1991) estimated that the food chain contributes about 97% of the total daily intake of B[*a*]P. The daily intake of B[*a*]P from foods in the USA is reported to be in the range of 0.16 to 1.6 μg/day (Santodonato *et al.*, 1981), which agrees well with the reported range for daily PAH intake of 1.1–22.5 μg *per capita* for the people in The Netherlands (Vaessen *et al.*, 1988). On the other hand, 100 cigarettes will produce similar amounts of B[*a*]P: 0.2–12.2 μg (IARC, 1973) or between 20 and 40 ng/cigarette (IARC, 1985). This means that smokers are exposed to additional B[*a*]P. Average smokers (i.e. 20 cigarettes/day) are taking in an additional 780 ng B[*a*]P daily, which means that smokers get an additional 16% B[*a*]P from smoking (Hattermer-Frey and Travis, 1991). Kramers and van der Heijden (1988) calculated that the maximum B[*a*]P uptake from ambient air is "in the order of 10-fold less than the estimated uptake via food", or equivalent to a mean of 10 cigarettes per day.

Lioy *et al.* (1988), measuring the total human exposure to B[*a*]P from inhalation in the home and foods, concluded that "the range of food exposures

Abbreviations: B[*a*]P = benzo[*a*]pyrene, PAHs = polycyclic aromatic hydrocarbons.

Table 1. *In vitro* extractability by water, ethanol and olive oil of B[*a*]P added to different foods

Type of food*	Percentage of radioactivity recovered in duplicate tests (average) following a single extraction step: Water	20% Ethanol	Olive oil
Chicken	7.0; 4.7 (5.9)	4.0; 4.4 (4.2)	76.8; 73.9 (75.4)
Pork	3.7; 3.8 (3.8)	3.1; 3.6 (3.4)	85.9; 89.2 (87.5)
Beef	14.4; 7.6 (11.0)	5.7; 6.6 (6.1)	88.9; 87.5 (88.2)
Corn	46.5; 45.6 (46.0)	25.7; 23.5 (24.6)	91.8; 94.3 (93.0)
Peas	26.9; 25.3 (26.1)	25.8; 26.4 (26.1)	92.9; 93.5 (93.2)
Apple	22.4; 22.6 (22.5)	19.9; 24.5 (22.2)	85.8; 86.6 (86.2)
Bran	3.1; 3.9 (3.5)	3.0; 2.3 (2.7)	71.9; 81.4 (76.7)

*All food samples were freeze-dried.
Samples were spiked with 230 ng [^{14}C]B[*a*]P/100 μl. For a description of the procedure see 'Procedure 1 (*in vitro*)' in the text.

was much greater than the range of air exposure". However, they found that in some homes, probably because of indoor combustion sources (e.g. cigarette smoke or coal-burning stove), the intake of B[*a*]P from food and air was quite similar.

Despite the apparently strong carcinogenic potential of these chemicals in tests with laboratory animals, no epidemiological evidence has been found to date linking their presence in foods to human cancers (Benford and Bridges, 1985; IARC, 1983). This indicates that, despite the presence of these mutagens/carcinogens in our food, humans are somehow 'protected' from the detrimental effects of these compounds. Because of the low levels of inducible *P*-450 isozymes in the intestinal tract, the ingestion of PAH-containing food rarely leads to tumours in the small and large intestine. However, the uptake of B[*a*]P from the gut is important in terms of its exposure to other organs by way of enterohepatic circulation.

A feeding study with laboratory animals is the best bioassay available to test the carcinogenic potential of foods or their contaminants, or to find out whether dietary 'protective' effects of some food ingredients exist. However, the induction of cancer at susceptible sites in laboratory animals depends on experimental conditions such as the amount of test compound administered, frequency and mode of administration, test chemical, vehicle, and the species and strain of the animal used (Stewart, 1967). When the test chemical is given orally, the type of diet and the content of other contaminants could also play an important role in the carcinogenic effects of the tested compound. Since food is a complex mixture, there is a chance that some food components may alter the uptake of a test compound, thereby inhibiting (Stich and Rosin, 1984; Wattenberg, 1985) or potentiating (Graham *et al.*, 1990; Howe *et al.*, 1990; Pariza, 1989) its toxic effects.

It appears that relatively little attention has been devoted to factors that affect the uptake of toxic agents in foods and/or to the possible interaction among them. A recent *in vitro* study indicates that hydrophobic mutagens can be adsorbed to some components of dietary fibre (Roberton *et al.*, 1990). Dietary factors may also form complexes with the toxic xenobiotics and thereby reduce their bioavailability (Sayer *et al.*, 1989).

In this paper we report the results of studies in which we investigated the uptake of B[*a*]P from different diets/media, and in which we considered the possible interaction between food flavonoids or regular food components and B[*a*]P in the alimentary tract. Since the main excretion route of B[*a*]P and other PAHs in mammals is bile (Chipman *et al.*, 1981), we used a bile-cannulated rat as an animal model to investigate the uptake of B[*a*]P from foods.

Table 2. *In vitro* extractability by water, oil and liquid paraffin of [^{14}C]B[*a*]P added to rat chow with or without carbon

Medium	Percentage of recovered radioactivity: Without carbon	With carbon added
Water*	1.7	0.5
	1.9	0.2
Olive oil*	84.6	0.7
	80	0.9
Liquid paraffin†	72.5	1.3
	71	1.3
	71.1	1.3

*Test done in duplicate.
†Test done in triplicate.
For a description of the procedure see 'Procedure 2A' in the text.

MATERIALS AND METHODS

Foods

The following freeze-dried foods were used in various *in vitro* or *in vivo* tests: green peas, cooked ground beef, boneless pork chops, diced carrots, cooked diced chicken and diced apples, all of which are commercially marketed by HarDee Farms Int. Ltd (Oakville, Ontario, Canada). Bran marketed by Quaker Oats Co. (Peterborough, Ontario, Canada) was also used.

Chemicals

The radioactive [7,10-^{14}C]B[*a*]P (sp. act. 52 mCi/mmol) was purchased from Amersham Canada Ltd (Oakville, Ontario, Canada); quercetin and chlorogenic acid were from Aldrich Chemical Co. (Milwaukee, WI, USA); and the activated carbon (grade G60) was manufactured by DARCO (Wilmington, DE, USA).

Laboratory animals

Sprague–Dawley male rats (Charles River, Montreal, Quebec, Canada), weighing about 300 g ($\pm$10%), were used for the bile cannulation. A group of four to six rats was used in each test, if not otherwise indicated.

Bile duct cannulation

All methods and procedures were approved by the Animal Care Committee and conducted under the supervision of a veterinarian. The basic surgical procedure for the bile duct cannulation has been well described previously (Chipman *et al.*, 1981). We found that after the surgery, the rat appeared more comfortable in a sling than if kept in a restrainer for many test hours (Stavric *et al.*, 1988). Bile duct cannulation was performed on rats anaesthetized with sodium pentobarbital. The cannula, made from polyethylene tubing (PE-10) (i.d. 0.28; o.d. 0.61 mm; Clay Adams, Parsippany, NJ, USA), was positioned through the right side of the abdominal wall. The free end of the cannula was pushed through the cloth sling that was used to hold the animal after surgery, and cut to a length of 20–25 cm.

After surgery, and throughout the test, the rats were kept in individual cloth slings, secured by clamps to a metal rod mounted on a laboratory stand. Four openings in the lower part of the sling were made for the rat's legs and the sling supported the entire lower part of the rat's body. By placing the cannulated rat in a sling, accidental removal of the cannula was prevented. Through an opening on the side of the sling, the cannula coming out of

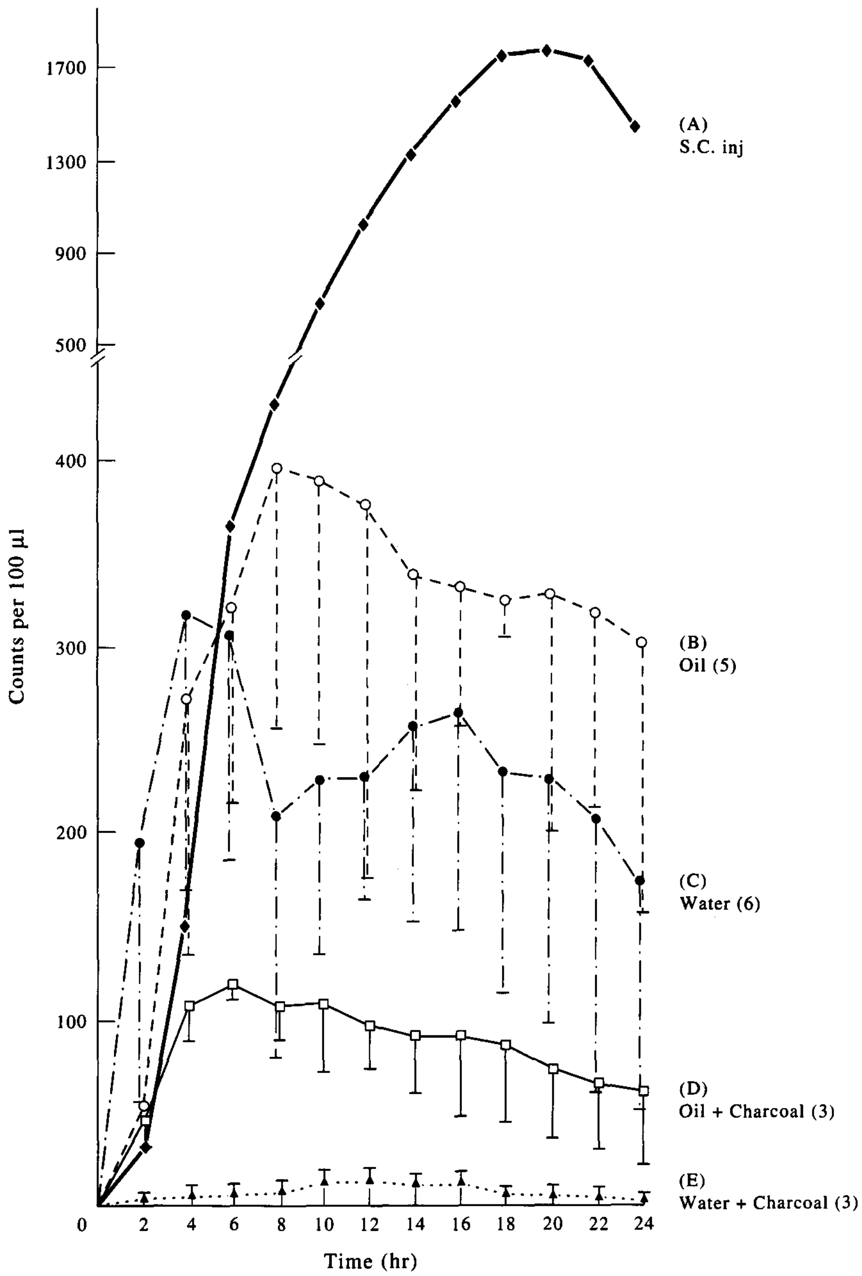

Fig. 1. Recovery of ^{14}C-radioactivity in the bile of rats given a single dose of 0.58 μg [^{14}C]B[*a*]P (200,000 cpm) orally in different media or subcutaneously in 20 μl toluene. Values are means for the number of rats per treatment given in parentheses, and range bars indicate the SD. One rat was treated with [^{14}C]B[*a*]P sc. For a description of the procedure used, see 'Procedure 2B (*in vivo*)' in the text.

Table 3. Bile volume, concentration and total recovery of radioactivity in the bile of bile duct cannulated rats within 24 hr after treatment with a single oral or sc dose [^{14}C]B[*a*]P

Media (mixture of) or vehicle	No. of rats	Route*	Bile volume (g/24 hr)†	Concentration of radioactivity (cpm/100 μl)†	Recovery of the Dose (%)†
Rat chow + water + carbon	4	Oral	19.7 ± 3.5	7.7 ± 1.3	0.7 ± 0.2
Rat chow + water	8	Oral	17.1 ± 2.6	89.4 ± 24.7	17.9 ± 7.5
Rat chow + oil + carbon	6	Oral	19.7 ± 2.1	226.8 ± 72.8	20.9 ± 11.7
Rat chow + oil	7	Oral	17.6 ± 4.0	292.7 ± 96.8	24.9 ± 6.7
Toluene solution	1	sc	18.5	1016	64.2

*The total volume of the oral doses was 1.5 ml; for the sc injection 20 μl of toluene was used as a vehicle.
†Values are means ± SD for groups of four to six rats. The procedure is described in the text as 'Procedure 2B (*in vivo*)'.

the abdominal incision was securely connected to a test-tube for the collection of bile, which was gravity fed. Another opening in the sling was made behind the rat's head for sc injections of 0.9% saline (2 ml/4 hr). This route for replenishment of the body fluids was found to be preferable to oral administration: the dose of the saline was controlled and there was no interaction between water and other orally introduced test ingredients (e.g. oil) once the test was in progress. This arrangement allowed free movement of the rat's head, legs and tail; thus the rats did not seem to be visibly stressed. Some were very active, so that a slight taping of the hind legs was occasionally necessary to prevent accidental removal of the cannula.

Procedure 1 (in vitro). These tests were performed with different freeze-dried foods obtained commercially, as listed above. The purpose was to follow the adsorption of B[*a*]P to different foods and its desorption using water, dilute ethanol or oil.

Samples of freeze-dried foods were thoroughly ground in a mortar and duplicate samples of 0.5 g pulverized material were transferred to 5-ml vials with screw caps. Each sample was then spiked at 230 ng/100 μl with [^{14}C]B[*a*]P dissolved in 95% ethanol (approximately 23,000 counts) and dispersed throughout the sample with an additional 1 ml chloroform. The vials were left uncovered until all of the chloroform had evaporated, which was determined by weight loss. To prevent any degradation or photooxidation of the [^{14}C]B[*a*]P all manipulations were performed in subdued light. The following day 3.0 ml water, 20% ethanol or corn oil was added to the vial and thoroughly vortexed for 1 min. After standing at room temperature for 1 hr, the vials were centrifuged at high speed (4750 rpm) using a clinical centrifuge. After centrifuging, 100 μl clear supernatant was removed for counting using Aquasol (NEN Research Products, DuPont, Boston, MA, USA) as medium.

Procedure 2 (in vitro and in vivo). These tests were performed using rat chow as a basic food with the purpose of investigating the effect of carbon. The desorption capacity for B[*a*]P in water, oil and liquid paraffin was investigated in *in vitro* (Procedure 2A) and *in vivo* (Procedure 2B) tests.

Finely pulverized rat chow (Purina Lab Chow M&R Feed, Pembroke, Ontario, Canada) as such (a), or thoroughly mixed with carbon at 1% (w/w) (b), was used for these tests. To a series of test-tubes, 0.5 g of chow (a) or (b) was added, and then each tube was spiked with [^{14}C]B[*a*]P, as described above. After evaporation of the organic solvents the spiked rat chow was homogenized in 3 ml extracting media (water, olive oil or liquid paraffin), and used either for *in vitro* extractability tests of B[*a*]P [Procedure 2A], or for *in vivo* tests with bile duct cannulated rats [Procedure 2B].

In Procedure 2A (*in vitro*), the samples were left at room temperature for 1 hr, and then centrifuged as described above. A 100-μl sample of clear supernatant was used for determining the radioactivity.

In Procedure 2B (*in vivo*), the homogenized samples were administered orally, by plastic gavage tubing (Suction Catheter, Argyle, ALOE, Brunswick Co., St Louis, MO, USA) to conscious, bile duct cannulated rats. Before bile duct cannulation the rats

Table 4. Effects of three dietary supplements on the recovery of ^{14}C radioactivity in the bile of bile duct cannulated rats given a single oral dose of [^{14}C]B[*a*]P*

Addition of rat chow	Bile volume (ml/14 hr)†	Concentration of radioactivity (cpm/100 μl)‡	Recovery of the dose (%)†
None (control)	12.0 ± 2.0	1099–2340	15.7 ± 4.3
Charcoal (0.5%)	11.4 ± 3.2	318 ± 712	3.1 ± 1.8
Charcoal (0.1%)	10.3 ± 2.3	994–1789	9.2 ± 3.6
Quercetin (2%)	11.9 ± 1.2	1122–2333	12.4 ± 5.4
Chlorogenic acid (2%)	11.0 ± 3.4	908–2606	13.2 ± 4.3

*Each rat was given orally 200,000 cpm [^{14}C]B[*a*]P.
†Values are means ± SD for groups of four rats.
‡Range. Bile was collected for 14 hr after dosing. Results are from all samples (seven collections of 2-hr bile samples) from each rat (four rats per group).
The procedure is described in the text as 'Procedure 3 (*in vivo*)'.

were fasted for 14 hr, with free access to water (Stavric *et al.*, 1988). After the intubation, the contents of the syringe and the tubing were washed with Aquasol and all washings were collected in a scintillation vial and the radioactivity was measured and subtracted from the original dose to determine the actual amount of [^{14}C]B[*a*]P given.

Procedure 3 (in vivo). These tests were performed to determine the uptake of B[*a*]P from food as such or from rat chow fortified with carbon or food polyphenols.

To minimize the individual variability due to different amounts of food in the stomach at the time of dosing following an overnight fast (Jeffrey *et al.*, 1987), attempts were made to ensure, as much as possible, that each rat had approximately the same amount of food in its stomach at the time of dosing. From 2 to 3 days before the surgical implantation of the cannula, the regular rat chow was replaced with the test diet (see below) until the afternoon of the day before the test (surgery). At that time, the rats were fasted overnight, with free access to water. The next morning the same test diet was offered again for 2 hr before the surgery, and the food consumption determined. After the bile duct cannulation had been performed and the animal had recovered from the anaesthesia, each rat was given an oral dose of [^{14}C]B[*a*]P (200,000 cpm in 1 ml 50% ethanol). The bile collection started immediately after the dosing (see below).

Using this schedule, the following diets were tested: regular pulverized rat chow (control); freeze-dried peas; freeze-dried beef; fortified rat chow containing (w/w) 0.1 or 0.5% carbon, 2% quercetin or 2% chlorogenic acid.

Collection of bile samples. After dosing, the bile was collected at 2-hr intervals for the next 14 or 24 hr in pre-weighed plastic test-tubes (11 × 75 mm). The weight of the sample was recorded, the sample was vortexed for 10 sec, and then a 100-μl aliquot was removed and placed in a scintillation vial. Aquasol (15 ml) was added, the vial was vortexed briefly, and the radioactivity was counted.

Radioactivity. The radioactivity in the bile samples, in cpm, was determined in a liquid BECKMAN LS 2800, scintillation counter (Beckman Instruments Inc., Fullerton, CA, USA).

Reporting of results. The *in vitro* results are reported as a percentage of the spiked dose of [^{14}C]B[*a*]P recovered in the medium supernatant (water, oil, 20% ethanol, or liquid paraffin).

The *in vivo* results were calculated in several ways: as the mean values $\pm$ SD of the total cpm per 2-hr sample; as a concentration of the radioactive dose (cpm per 100 μl of bile) in each sample; as the percentage of the given radioactive dose recovered in 2-hr samples of bile; or as the total recovery of radioactivity for 14 or 24 hr after dosing.

RESULTS

Tables 1 and 2 indicate the percentage of extracted radioactivity in the *in vitro* tests. Both tables illustrate the large differences between the three solvents in B[*a*]P extractability depending on the food, and the possible importance of carbon in reducing the amount of B[*a*]P available for intestinal absorption. From the rat chow the olive oil extracted more than 80% of the spiked [^{14}C]B[*a*]P, whereas water extracted less than 2%. Extraction by liquid paraffin was similar to that by olive oil (Table 2). Table 2 confirms the previously observed (Table 1) lower extractability of B[*a*]P by water in comparison with oil. However, this experiment also illustrates that different foods possess a different capacity for adsorption or desorption of B[*a*]P. For all seven tested foods (Table 1), oil extracted much more B[*a*]P than water; for some foods (e.g. bran or pork) the difference was 20–25 times greater. The smallest difference was found for corn, where only a two-fold difference was observed.

Figure 1 illustrates the recovery of radioactivity (cpm) in the bile of rats orally treated with [^{14}C]B[*a*]P and the effect of water and oil on its intestinal uptake. Concurrent administration of carbon markedly reduced the elimination of radioactivity in the bile regardless of whether the ^{14}C-labelled B[*a*]P was administered in water or oil. This Figure also illustrates the excretion profile of radioactivity in the bile of a rat treated sc with [^{14}C]B[*a*]P.

Table 3 shows the recoveries of the radioactivity in the bile for the first 24 hr after dosing (Procedure 2B *in vivo*). The lowest recovery was found in the bile of rats given rat chow containing carbon and homogenized in water, for which only 0.75% of the dose was

Table 5. The effect of freeze-dried peas and beef on the uptake of B[*a*]P from the intestine of rats during 14 hr after dosing, as measured by the ^{14}C-radioactivity recovered in the bile of bile duct cannulated rats given a single oral dose of [^{14}C]B[*a*]P*

Food	No. of rats	Radioactivity recovered in the bile (cpm × 1000)†	Recovery of the dose (%)
Rat chow (control)	4	25–44	15
Peas (freeze-dried)	3	12–34	12
Beef (freeze-dried)	3	17–23	10

*Each rat was given orally 200,000 cpm [^{14}C]B[*a*]P.
†Range. Bile was collected for 14 hr after dosing. Results are from all samples (seven collections of 2-hr bile samples) from each of the rats in the treatment group.
The procedure is described in the text as 'Procedure 3 (*in vivo*)'.

recovered. The best recovery, about 25%, was when B[*a*]P was introduced in an oil medium. The rat that was injected sc with [^{14}C]B[*a*]P excreted 64.2% of the dose in the bile during the first 24 hr.

Table 4 summarizes the effect of four different dietary supplements (Procedure 3 *in vivo* studies) on the absorption of B[*a*]P and its recovery in bile within 14 hr of dosing. Whereas quercetin and chlorogenic acid produced modest reductions in the elimination of B[*a*]P or its metabolites, carbon had a substantial effect.

Table 5 summarizes the results of tests in which the bile cannulated rats were fed rat chow or freeze-dried samples of peas and beef, before being given an oral dose of [^{14}C]B[*a*]P (Procedure 3). Although a limited number of rats was used, this test indicated that peas and beef do reduce the absorption of B[*a*]P.

DISCUSSION

Physical or chemical interactions are known to occur between food constituents in a meal, which could change the availability of nutrients as well as xenobiotics. Food has been shown to influence the bioavailability of orally administered drugs (Welling, 1989). Of special interest for this report are possible interactions of common food constituents with xenobiotics formed as pyrolysed products during the preparation of food, such as heterocyclic aromatic amines or PAHs like B[*a*]P. In *in vitro* tests these xenobiotics are potent mutagens; however their mutagenic potency is reduced in the presence of some plant flavonoids or other food components (Alldrick *et al.*, 1986; Busk *et al.*, 1982; Stavric *et al.*, 1990). It is essential that these inhibitory effects from the *in vitro* tests are confirmed in *in vivo* studies. However, in *in vivo* studies there are other factors that could exert an influence on the results with regard to the 'beneficial' effect of certain food constituents towards some xenobiotics.

The results of the work described here indicate that diet or some specific components in food could play a significant role in influencing and reducing the bioavailability of B[*a*]P. Any food seems to retard the availability or the uptake of B[*a*]P from the gastrointestinal tract. Carbon, a possible food component, appears to be the most effective of those tested. The results of the *in vitro* tests, which showed that carbon firmly adsorbs B[*a*]P, were confirmed by the *in vivo* experiments in which the availability of B[*a*]P was significantly reduced by carbon. Therefore the presence of carbon could be one of the 'protective' factors in reducing the absorption of PAHs from barbequed or 'well-done' steaks. *In vitro* tests also indicate that about 50 times more B[*a*]P was extracted from food by oil than by water. The results suggest that water, in which B[*a*]P is not soluble, may reduce the transfer of B[*a*]P from the food particles to the intestinal mucosa. In contrast, the oil, in which B[*a*]P is soluble, facilitated transfer of B[*a*]P into oil (*in vitro* test) and *in vivo* the oil possibly enhanced B[*a*]P transfer to the intestinal wall. It is too early to speculate whether this could be one of the reasons why people who consume foods containing lipophilic xenobiotics and high levels of fat are prone to a higher cancer incidence.

Flavonoids and polyphenols (e.g. quercetin or chlorogenic acid) have been shown to affect the biological activity of known carcinogens in studies using animal models (for review see Stavric and Matula, 1988). The results of the present study suggest that the food polyphenols, at least partly, reduce the intake of B[*a*]P (and maybe of some other xenobiotics) from the intestine. This is in agreement with our results from the *in vivo* test with intact mice, orally dosed with B[*a*]P and quercetin or chlorogenic acid, which suggested that flavonoids prevented the absorption of B[*a*]P from the intestine (Stavric *et al.*, 1990).

We expected that peas, because of their polyphenol and fibre content, would have a greater capacity than beef to interfere with B[*a*]P and reduce its availability from the gastro-intestinal tract. However, both the *in vitro* (Table 1) and *in vivo* (Table 5) results, indicated that beef by itself has a capacity to adsorb B[*a*]P and/or its metabolites and make it/them less available for absorption.

When a standard dose of [^{14}C]B[*a*]P was administered to the rat by sc injection, thereby completely avoiding the gastro-intestinal tract, the recovery of radioactivity in the bile during the first 24 hr after dosing was 64%, in comparison with recoveries from less than 1% up to 25% when the [^{14}C]B[*a*]P was introduced orally with samples of foods (Tables 3–5). If the collection of bile from the sc treated rat had continued for several more hours, the recovery would probably have been much greater (see Fig. 1). This information gives support to the concept that foods play a role in restricting or delaying the absorption of B[*a*]P. Our results are in agreement with work by Jagadeeson and Krishnaswamy (1989), who reported a significant increase in the binding of B[*a*]P to hepatic DNA in food-restricted animals.

Besides the influence of the hydrophilic or lipophilic media (e.g. water or oil) for the desorption and transfer of B[*a*]P from food particles to the intestinal mucosa, there are several other possible mechanisms that may reduce the intestinal uptake of B[*a*]P. It appears that during gastro-intestinal digestion some food components (e.g. ellagic acid) may form new complexes with B[*a*]P and its metabolites, rendering them biologically inactive or non-absorbable (Sayer *et al.*, 1989). Other food components (e.g. fibre, polyphenols) could adsorb B[*a*]P very strongly (Howard and Fazio, 1980). In either case, the level of free xenobiotics available for uptake from the gastro-intestinal tract will be reduced. The adsorption of B[*a*]P onto ingested foods could be so firm that normal extraction procedures may fail quantitatively to elute B[*a*]P from the faecal material of rats gavaged with ^{14}C-labelled B[*a*]P (Hecht *et al.*, 1979). We observed similar difficulties in extracting the radioactivity from the faeces of rats dosed with [^{14}C]B[*a*]P-spiked rat chow. After several extended extractions with organic solvents, only 50% of the radioactivity from the faecal material was recovered. The total amount of radioactivity in the faeces was established indirectly, by measuring the amount of $^{14}CO_2$ after the combustion of the same faecal samples. The total recoveries of the given radioactivity (bile + faeces) were in a range of 80 to 90% (results not shown).

In this paper we report the results of several different tests, and although some of them used only a few animals, the results support the general consensus that food or some components in food can considerably influence the bioavailability of B[*a*]P and probably of other PAHs. This statement is also supported by the results of another set of studies, using the host-mediated assay and mice. It was found that the effect of plant flavonoids in reducing the mutagenicity of B[*a*]P *in vivo* was due not only to interference with enzymatic processes, but also to their interference with the absorption of B[*a*]P from the intestine (Stavric *et al.*, 1992).

Although in this experiment we did not analyse the rate of formation of metabolites or the relative amount of B[*a*]P in the general circulation, previous extensive work from many investigators indicates that once it has been absorbed or has been injected into the bloodstream, B[*a*]P is rapidly (in minutes) cleared from the blood (IARC, 1983). Mammary and other fat tissues are storage depots for PAHs from where they may be released slowly. The highest levels of hydrocarbons or their metabolites are concentrated in the gut (IARC, 1983). Renal excretion accounts for only 1–3% of the given dose of B[*a*]P (Chipman *et al.*, 1981; Foth *et al.*, 1988; Hecht *et al.*, 1979). The main elimination pathway for B[*a*]P proceeds through the bile followed by enterohepatic circulation. Therefore, the major route of elimination for B[*a*]P is the faeces.

Consequently, by using the bile duct cannulated rat and ^{14}C-labelled B[*a*]P added to some foods, it was possible to monitor the elimination pattern of B[*a*]P and/or its metabolites through the bile, which in turn indicated the uptake of B[*a*]P from different dietary media. Our results indicate that food could play a significant role in influencing the availability of B[*a*]P from the gut by altering its absorption. It appears that 'oily' foods facilitate the transfer of B[*a*]P from food particles to the intestinal walls, whereas 'watery' foods reduce this transfer. In addition, some common components of green vegetables (i.e. quercetin and chlorogenic acid) inhibit the availability of B[*a*]P, whereas carbon strongly adsorbs B[*a*]P, thereby decreasing its bioavailability.

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